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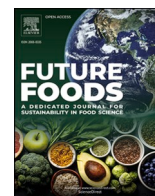
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Sorghum protein ingredients: Production, compositional variability and enhancement of aqueous dispersibility through homogenization

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ABSTRACT

Sorghum protein ingredients show promising applications, given that sorghum is a resilient grain with high protein content and bioactive components, such as tannins, in certain genotypes. This study aimed to investigate the production and characterization of sorghum protein ingredients extracted from both tannin-free and tannin-rich cultivars, along with an examination of their behavior in aqueous suspensions. The use of high-pressure homogenization to improve ingredient dispersibility was also assessed. Protein extracts exhibited protein contents ranging from 50 to 67 g/100 g (wet basis), with the highest values obtained in tannin-free samples. Total lipid contents were between 18 and 26 g/100 g (wet basis), with a high contribution of free fatty acids (68–76 g/100 g lipids), and tocopherol contents ranged from 1080 to 2039 µg/g total lipids. This is substantially higher than the lipid content in flours, implying an accumulation of lipids during the protein extraction process. While the dispersibility of the protein extracts in aqueous media was initially limited, high-pressure homogenization proved effective in reducing the average size of the particles in suspension, from 9–66 µm to 1.8–2.5 µm. This processing step significantly enhanced protein dispersibility by up to 288 %, especially for the tannin-rich samples.

1. Introduction

Sorghum (*Sorghum bicolor* L.) is a versatile grain commonly cultivated in arid regions of Africa, Asia, Australia, and North and South America (Girard and Awika, 2018), with a profitable growing owing to its adaptability to various environmental conditions. Sorghum was the sixth most produced cereal in the world in 2020, with a total production of almost 59 million tons (FAOSTAT, 2022). In semi-arid zones of Africa, South America, and Asia, sorghum is consumed as a staple food (Proietti et al., 2015; Queiroz et al., 2011), but its production is mainly intended for industrial conversion into alcohol and for animal feed (Liu et al.,

2018). In some countries, such as Japan, the United States and Brazil, this grain has been studied and recommended as a good alternative for food applications. Sorghum grains are mainly composed of carbohydrates (75 g/100 g) and their dietary fiber content is about 6 g/100 g. Lipids account for approximately 3 g/100 g, with a typical main fatty acid composition as follows: oleic acid (31.1 - 48.9 %), linoleic acid (27.6 - 50.7 %) and palmitic acid (11.7 - 20.2 %) (Mehmood et al., 2008).

Sorghum grains serve as an important protein source, with a content ranging from 6 to 18 g/100 g (total basis) (de Mesa-Stonestreet et al., 2010). This underscores the potential for using sorghum proteins as a

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sustainable alternative to animal proteins and as a natural ingredient in foods, which is of interest given the ongoing protein transition and prevailing clean label trend (Vallons et al., 2010). The production of sorghum protein-rich ingredients, as well as protein ingredients in general, requires an extraction process (i.e., separation of the proteins from the other components present in the seeds or grains, such as starch, fibers and antinutritional compounds). A comprehensive characterization of the final ingredient is crucial for assessing the impact and efficiency of the extraction process. Moreover, this characterization can provide insights into how non-protein compounds may influence the functional properties of plant-based protein-rich ingredients (e.g., emulsifying, gelling and mousing properties, and interfacial behavior) (Keuleyan et al., 2023).

Some sorghum cultivars exhibit a significant polyphenol content, including condensed tannins (procyanidins) (Barros et al., 2014). These tannins have the capacity to bind to proteins and form insoluble complexes. While this interaction is often considered a reason to label tannins as antinutritional factors, their potentially adverse effects may be offset by other positive functionalities, such as their antioxidant potential. Among the proteins present in sorghum grains, the most abundant is kafirin, belonging to the family of prolamins. Kafirin shares similarities with zein in solubility, molar mass, amino acid composition and polypeptide structure, although it is more hydrophobic (Xiao et al., 2015). In terms of molar mass, kafirin can be classified into α -kafirin (66–80%), with molecular weights of 23 and 25 kDa, β -kafirin (5–8%) and γ -kafirin (9–12%), with molecular weights of 18 and 28 kDa, respectively (Espinosa-Ramírez and Serna-Saldívar, 2016; Xiao et al., 2017).

The low water solubility of sorghum proteins can be a problem for their application in food products; therefore, dedicated strategies should be implemented to facilitate their dispersibility in aqueous media, which implies some structural modifications. For instance, high-pressure homogenization can be used to modify both the size and structural organization of the protein-based supramolecular assemblies (aggregates, particles) in suspensions. This mechanical treatment induces an intense shear on the protein suspension by forcing it to pass through a small gap, which can disrupt protein aggregates (Balny and Masson, 1993; Zamora and Guamis, 2015). Hence, the application of high pressure homogenization can enhance the techno-functionalities of proteins (Bader et al., 2011), such as their emulsifying properties. This underscores the significance of this processing step as a relevant pre-treatment for plant-based proteins (Burger and Zhang, 2019; Keuleyan et al., 2023; Levy et al., 2022; Melchior et al., 2022).

This study therefore aimed to characterize the composition of protein ingredients prepared from four sorghum cultivars with contrasted tannin content. Additionally, the impact of high-pressure homogenization on the particle size and protein dispersibility in aqueous suspensions prepared from these ingredients was investigated. Thus, this work represents an important step in facilitating the future utilization of this relevant crop in food products.

2. Materials and methods

2.1. Samples and reagents

Flours of four different sorghum cultivars (two cultivars without tannins, BR501 and BRS310, and two cultivars rich in tannins, BRS305 and SC782) were provided by Embrapa Milho e Sorgo (Sete Lagoas, MG, Brazil). For clarity, the suffixes (T-) and (T+) are added to the sample codes to recall the absence or presence of endogenous tannins, respectively.

Ethanol, sodium metabisulfite and sodium hydroxide were from Labsynth (Diadema, Brazil). Anhydrous sodium sulfate, toluene and cyclohexane were from Carlo Erba Reagents (Val-de-Reuil, France). Dichloromethane, methanol and chloroform were from Biosolve Chemicals (Dieuze, France). Phloroglucinol, heptadecanoic acid (C17:0), boron trifluoride-methanol (BF₃) and Nile Red were from

Sigma-Aldrich (St Louis, USA). Standards of α , β , γ and δ -tocopherol were from Calbiochem Item from Merck and the γ -tocotrienol standard was from Cayman Chemical (Ann Arbor, USA). Sodium acetate and β -mercaptoethanol were from Merck (Darmstadt, Germany). Ascorbic acid was from Fisher Scientific (Loughborough, UK), sodium chloride was from VWR International (Radnor, USA) and Alexa 488 was from Invitrogen, Thermo Fisher Scientific (Waltham, USA). All the chemicals were of analytical grade and ultrapure water was used for all the experiments.

2.2. Production of protein extracts

Protein-rich fractions were extracted from the sorghum flours following the methodology described by Taylor et al. (2005). Briefly, each flour was dispersed in an aqueous solution containing 70% ethanol (v/v), 0.5% sodium metabisulfite (w/v) and 0.35% NaOH (w/v), at 70 °C for 1 hour, under constant stirring, at a ratio of 1:5 (w/w, flour: aqueous phase), followed by centrifugation at 1000 × g for 5 min at 25 °C (Hitachi CR22N, Tokyo, Japan). The supernatant was collected and placed overnight in a fume cupboard, at room temperature (25 °C) for solvent evaporation until a viscous liquid sediment was formed. The protein slurry was added to cold distilled water (< 10 °C) and the pH was adjusted to 5.0 with HCl 1 M to precipitate the proteins. Proteins were recovered by filtration (14 μ m) under vacuum, collecting the material on the top of the filter and dispersing it in distilled water to neutralize (7.0) the pH with NaOH 1 M before subsequent freeze-drying. Contrarily to the reference procedure described by Taylor et al. (2005), the starting flours were not defatted with hexane in order to evaluate the functional properties of protein-rich extracts in the presence of lipids, which is relevant given the current incentives for minimal fractionation routes.

2.3. Preparation of protein suspensions

Suspensions containing 2% (w/w) of protein extracts were prepared in ultrapure water. The suspensions were stirred overnight at 4 °C to ensure maximum hydration. The suspensions were then treated by high pressure homogenization (HPH) at 400 bars for 6 min, corresponding to 24 passes through the homogenizer (Panda plus 1000, GEA, Italy) in one continuous process. These conditions were preliminary optimized to have a particle size and distribution fairly comparable among the different samples (Supplementary Fig. 1).

2.4. Characterization of sorghum flours and freeze-dried protein extracts

2.4.1. Proximate composition of the powders

Flours and protein extracts were characterized according to AOAC (2006) official methods for protein, water and ash contents.

The protein content was evaluated by the Kjeldahl method, and the nitrogen-to-protein conversion factor (N:P factor) was computed as the ratio of total anhydrous mass of amino acids to the total mass of nitrogen (Sosulski and Imafidon, 1990). Asparagine (Asn) and glutamine (Gln) were assayed in their acidic form, i.e., as asparagic acid (Asp) and glutamic acid (Glu). The relative contents in Asp, Asn, Gln and Glu were estimated using kafirin sequences (UniProt reference: P14690, P14691 and P14692).

Moisture content was analyzed by measuring the mass of water removed during drying in a vacuum oven (Marconi MA030, Brazil) at 60 °C for 48 h. The ash content was evaluated by incineration in a muffle furnace (Quimis Q318S25T, Brazil) at 550 °C until the residue was white or light gray. The total carbohydrate content, including fibers, was calculated by weight difference (FAO, 2003).

2.4.2. Quantification of total flavanols

The quantification of total flavanols (flavanol monomers (catechin and epicatechin) and procyanidin oligomers and polymers (i.e., condensed tannins)) was performed after acidolysis in the presence of

phloroglucinol and analysis of the reaction media by UHPLC (Waters) coupled to UV–visible diode array detection (Waters) and mass spectrometry (TQD Quattro Waters) according to a procedure adapted from Malec et al. (2014). Aliquots of sorghum flours or protein extracts were weighed (100 mg for tannin-free samples BR501(T-) and BRS310(T-), and 30 and 10 mg for tannin-rich samples BRS305(T+) and SC782(T+), respectively) and freeze-dried before further analysis. The freeze-dried samples were mixed with 400 μ L methanol/HCl 0.3 N and 800 μ L of a mixture containing phloroglucinol (75 g/L) and ascorbic acid (15 g/L). The mixture was immediately homogenized by sonication and incubated for 30 min at 50 °C (Fisher Scientific, Iowa, USA), with intermittent stirring at 10 and 20 min. The tubes were placed in an ice bath for 5 min, stirred again, and added with 1.2 mL sodium acetate (0.2 M) to stop the reaction, following by new stirring and centrifugation at 8000 \times g for 5 min at 19 °C (Thermo Fisher Scientific Sorvall LYNX 6000, Germany). The supernatants were collected, placed in a VivaSpin tube (Reference: VS02H01, Membrane: 10.000 MWCO HY) and centrifuged at 8000 \times g for 30 min at 19 °C (Thermo Fisher Scientific Sorvall LYNX 6000, Germany). Then, the filtrates were analyzed by UHPLC (Waters). This procedure was performed in triplicate.

2.4.3. Lipid extraction and composition analysis of the powder samples

Total lipids were extracted as described by Fogang Mba et al. (2018). Briefly, after hydration of the powder in ultrapure water overnight at 4 °C under magnetic stirring, dichloromethane/methanol (2:1, v/v) was added, the system was stirred on a vortex, centrifuged at 1700 \times g for 10 min at 20 °C (Eppendorf 5810 R, Germany) and the lower organic phase was recovered in a volumetric flask. This procedure was repeated two more times, and the lower phases were pooled in the same volumetric flask. The mixture was put in a separation funnel with the addition of 0.73 % (w/v) sodium chloride solution and stored at 4 °C overnight. The lower phase was recovered in another volumetric flask, previously weighed, using a hopper with glass wool and anhydrous sodium sulfate. The solvent was evaporated in a rotative evaporator (R-100, Rotavapor, Büchi, France) at 40 °C and the sample was dried under nitrogen flow (N-Evap 111, Organomation, USA). The lipid content was calculated by weight difference (Eq. (1)) and expressed in g lipids/g sample. Chloroform was added to the volumetric flask and the lipid extracts were stored at –80 °C until subsequent analysis of lipid classes, fatty acid composition, and tocopherols.

$$\text{Lipid content} = \frac{\text{mass of volumetric flask with lipids} - \text{mass of empty volumetric flask}}{\text{mass of sample}} \quad (1)$$

Lipid classes (neutral lipids – triglycerides, diglycerides, monoglycerides and free fatty acids – and polar lipids – phospholipids) were analyzed by high performance liquid chromatography (HPLC). Approximately 5 μ g lipid extract in 10 μ L chloroform were analyzed with a modular UltiMate 3000 RS system (Dionex, Voisins Le Bretonneux, France) equipped with an Uptisphere Strategy column (150 mm \times 4.6 mm, 2.2 μ m, 100 Å; Interchim, Montluçon, France) and coupled to an evaporative light scattering detector (ELSD) Sedex 85 (Sedere S.A., Alfortville, France). Chromatographic conditions, identification and quantification of lipids were as described by Fogang Mba et al. (2017).

For the determination of the fatty acid composition, the lipid extracts were first derivatized to fatty acid methyl esters (FAMES) according to Morrison and Smith (1964). For this, 100 μ L of lipid extract (i.e., around 1 mg lipids) were mixed with 100 μ L of internal standard solution (C17:0, 1 mg/mL in chloroform) in a glass tube and the solvent was

evaporated under nitrogen flow (N-Evap 111, Organomation, USA). Then, 1 mL toluene and 1 mL BF₃ reagent at 14 % in methanol were added and the mixture was incubated at 100 °C for 45 min in a dry bath (Fisher Bioblock Scientific, Ilkirch, France). After cooling, 1 mL cyclohexane and 0.5 mL ultrapure water were added, and the mixture was vortexed for 20 s. After centrifugation at 900 \times g for 5 min at 20 °C (Eppendorf 5810 R, Germany), the upper phase containing cyclohexane and FAMES was recovered and analyzed by a gas chromatograph (Clarus 680, Perkin Elmer, Shelton, USA) on a capillary column (DB 225MS, 30 m \times 0.32 mm, film thickness 0.25 μ m, Agilent Technologies, USA) according to the procedure described by Meynier et al. (2014). The identification of individual fatty acids was made by comparing the retention time of these components with those of a standard mixture (FAME mix; Supelco, Sigma Saint Quentin Fallavier, France) and the peak areas were integrated and corrected by the response factor of each individual fatty acid (Ackman, 2007). The results were expressed in mg fatty acids/g lipids.

The tocopherol content was measured by HPLC (UHPLC Ultimate 3000 system, Thermo Fisher Scientific, Germany) as described by Meynier et al. (2014) with slight modifications. Briefly, 40 μ L lipid extract were injected onto a normal phase column (Acclaim Polar Advantage II, Dionex, Si 3 μ m, 250 mm \times 3 mm) and the separation was achieved in isocratic mode. Hexane/tert-butyl-ether (90/10 v/v) was used as the mobile phase at a flow rate of 0.5 mL/min, and 295 nm and 330 nm were used as excitation and emission wavelengths for the fluorescence detector, respectively. The identification and concentration of the tocopherol classes in the samples were determined comparing their retention time with those of standard compounds and using external calibration curves of tocopherol isomers and γ -tocotrienol from commercial standards, respectively. The results were expressed in μ g tocopherols/g lipids.

2.4.4. Fourier transform infrared (FTIR) spectrophotometry

A FTIR spectrophotometer Thermo Nicolet IS50 (Thermo Scientific, USA) connected to an attenuator of total reflectance (FTIR-ATR) with deuterated triglycine sulfate (DTGS) was used to obtain FTIR spectra for the sorghum flours, the freeze-dried protein extracts and the protein suspensions produced thereof. Powdered samples and protein suspensions were placed in a single-bounce diamond crystal and a ZnSe crystal, respectively, and 50 scans were collected in the range of 4000 to 650

cm⁻¹, with 4 cm⁻¹ resolution.

2.4.5. Protein composition analysis

The protein composition in the flours, protein extracts and protein suspensions was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A size exclusion - high performance liquid chromatography (SE-HPLC) assay was conducted for both flours and their corresponding protein extracts.

For analyzing the powders by SDS-PAGE, total kafirins were extracted from raw flour according to D'Almeida et al. (2021) and the protein solutions were freeze-dried before being dispersed at 1.5 g/mL during 15 min at 70 °C in 10 mM borate pH 10.0 buffer containing 2 % SDS and 20 mM dithioerythritol (DTE). Equivalents of 12 μ g of protein/sample were loaded on a pre-casted gel and run for 2 h using a Bio-Rad Mini Protean® 3 Cell (USA) system. Pre-stained protein markers with molecular weights ranging from 10 to 250 kDa (Biorad) were loaded in a 4–20 % gradient gel (Biorad). Then, the gel was stained and decolorized following the method described by Neuhoﬀ et al. (1988).

For SDS-PAGE analysis of the protein suspensions and of their supernatants after centrifugation ($10,000 \times g$ for 30 min at 10°C), samples were first denatured and reduced by heating at 95°C for 10 min in a buffer solution containing 62.5 mM Tris-HCl buffer (pH 6.8), 2% (w/v) SDS, 25% (v/v) glycerol, 0.01% bromophenol blue and 5% (v/v) β -mercaptoethanol (β -ME). Pre-stained protein markers (SeeBlue, Novex, Life technologies, Thermo Fisher, France), with molecular weights ranging from 3 to 198 kDa, and 5 μL of each sample were loaded in an 8–16% gradient gel. Migration was conducted for 2 h using a Bio-Rad Mini Protean® 3 Cell (USA) system. Subsequently, the gel was exposed to a brilliant blue Coomassie G 250 solution under gentle agitation (Rocker 25, Labnet, Labnet International Inc.) for 2 h. Following this, it was rinsed with distilled water and subjected to scanning and analysis using Multi Gauge V3.0 software (Fujifilms).

For SE-HPLC analysis of the powders, the procedure was carried out as described by Morel et al. (2022), with some modifications. In brief, a protein extraction step was performed to recover soluble proteins, by dispersing 50 mg powder (flour or protein extract) in 1 mL 10 mM Na-borate buffer pH 10.0, SDS 2%. The mixture was stirred for 20 min at 20°C , and centrifuged at $20,000 \times g$ for 10 min at 20°C (Eppendorf 5427 R, Germany). The supernatant was then recovered and added into a new tube, repeating this procedure two more times. The pellet resulting from the centrifugation was dispersed in 1 mL of the same buffer complemented with 20 mM of DTE, following the same parameters as described above for recovery of the insoluble proteins. The protein analysis was performed at 25°C , on an Alliance Waters HPLC chain equipped with a TSK G4000 (Tosoh bioscience) (7.8×300 mm) column and a TSK gel SW_{XL} (4 cm \times 6 mm) pre-column, eluted with the phosphate buffer, 0.1 M, pH 6.8, SDS 0.1%. A volume of 15 μL was injected and eluted at 0.7 mL/min; the detection of different proteins was done at 214 nm. The data were collected with the help of Empower software. The mass calibration of the device was carried out by injecting a standard protein of known molecular weight and the area under the curve was integrated, using a response coefficient determined experimentally, to determine the protein content.

2.4.6. Colloidal morphology of the protein suspensions

The particle size distribution and mean volume diameter ($d_{4,3}$) in the aqueous suspensions were determined by static light scattering (Horiba LA-950, Japan). The refractive indices were set to 1.450 for the dispersed (protein) phase and 1.333 for the continuous (aqueous) phase.

Particle morphology was evaluated by optical microscopy (Zeiss Axioskop, equipped with Prosilica Digital Camera 5DCAM 1.31, and image software), and confocal laser scanning microscopy (CLSM) (Inverted Nikon A1 laser scanning confocal microscope) using Alexa 488 and Nile red to stain the proteins and the lipids, respectively, as described by Keuleyan et al. (2023).

2.4.7. Solubility of protein extracts in aqueous media

Suspensions, both treated and non-treated by HPH, were analyzed before and after centrifugation at $10,000 \times g$ for 30 min at 10°C (Eppendorf 5810 R, Germany) regarding the nitrogen content in the supernatant using the Dumas method (Rapid MAX N exceed, Elementar, Germany). The protein solubility (S) was calculated using Eq. (2).

$$S = \frac{\text{Nitrogen content}_{\text{supernatant}}}{\text{Nitrogen content}_{\text{suspension}}} \times 100\% \quad (2)$$

2.5. Statistical analysis

Results obtained from triplicate analytical determinations underwent analysis of variance (ANOVA) and differences between means were assessed by the Tukey test at 5% probability level (XLStat).

3. Results and discussion

3.1. Characterization of the samples

3.1.1. Proximate composition of the powder samples

The starting flours displayed protein contents ranging from 9.6 to 10.6 g/100 g (wet basis, w.b.) (Table 1), with no systematic effect of the tannin content. Specifically, samples BR501(T-) and SC782(T+) did not exhibit a significant difference, while a notable distinction was observed between samples BRS310(T-) and BRS305(T+). Martino et al. (2012) studied eight Brazilian sorghum cultivars, developed and cultivated by Embrapa Milho e Sorgo (Sete Lagoas/MG) and obtained protein contents ranging from 8.6 to 11.9 g/100 g (w.b.), aligning closely with the findings of the present study. The cultivars BR501(T-), BRS310(T-) and BRS305(T+) were also analyzed by Martino et al. (2012), yielding slightly different results for protein content. Antunes et al. (2007) explored the genotype BR501(T-) and reported approximately 11.2 g/100 g (dry basis, d.b.) of protein, equivalent to 10.1 g/100 g (w.b.). This falls within the range observed in the present work and the results obtained by Martino et al. (2012).

The protein content in the extracts ranged from 55 to 67 g/100 g (w.b.) (Table 1), with a significantly lower content for the (T+) extracts compared to the (T-) extracts. This suggests that the presence of tannins tends to decrease the protein content after the extraction process, possibly due to the ability of tannins to covalently and irreversibly bind to proteins, hampering their extractability (Duodu et al., 2003). Using a similar sorghum protein extraction method, Taylor et al. (2005) reported protein contents in the extracts ranging from 74.6 to 89.3 g/100 g (d.b.), before and after the defatting process, respectively. In a study by Da Silva and Taylor (2004), employing the same reagents with slight differences in the extraction process, protein contents ranging from 77.9 to 83.1 g/100 g (d.b.) were obtained without the defatting process, and approximately 88 g/100 g (d.b.) after defatting with hexane.

Table 1

Composition (g/100 g total basis) of sorghum flours and their protein extracts. BR501(T-) and BRS310(T-) are the cultivars without tannins, whereas BRS305(T+) and SC782(T+) are the cultivars with tannins.

Sample		Proteins ¹	Total lipids	Water	Ash	Total procyanidins	Carbohydrates (including fibers) ²
Flours	BR501(T-)	10.30 \pm 0.16 ^{ef}	3.51 \pm 0.03 ^d	8.27 \pm 0.03 ^a	1.40 \pm 0.03 ^a	n.d. ³	76.52 \pm 0.09 ^a
	BRS310(T-)	10.60 \pm 0.24 ^e	3.04 \pm 0.20 ^{de}	8.18 \pm 0.01 ^a	1.38 \pm 0.03 ^{ab}	n.d.	76.80 \pm 0.15 ^a
	BRS305(T+)	9.64 \pm 0.09 g	3.12 \pm 0.16 ^{de}	8.19 \pm 0.03 ^a	1.29 \pm 0.02 ^c	2.06 \pm 0.00	75.70 \pm 0.13 ^a
	SC782(T+)	10.10 \pm 0.18 ^f	2.58 \pm 0.29 ^e	8.04 \pm 0.02 ^b	1.20 \pm 0.04 ^{de}	1.82 \pm 0.00	76.26 \pm 0.18 ^a
Protein extracts	BR501(T-)	65.22 \pm 0.56 ^b	22.19 \pm 0.45 ^b	4.30 \pm 0.07 ^c	1.41 \pm 0.01 ^a	n.d.	6.88 \pm 0.71 ^d
	BRS310(T-)	67.14 \pm 0.35 ^a	21.88 \pm 0.12 ^b	3.73 \pm 0.06 ^d	1.35 \pm 0.01 ^b	n.d.	5.90 \pm 0.64 ^e
	BRS305(T+)	54.99 \pm 0.37 ^c	25.84 \pm 0.13 ^a	3.39 \pm 0.03 ^f	1.27 \pm 0.04 ^{cd}	1.27 \pm 0.00	13.24 \pm 0.51 ^c
	SC782(T+)	50.08 \pm 1.46 ^d	18.71 \pm 0.63 ^c	3.59 \pm 0.03 ^e	1.15 \pm 0.02 ^e	1.95 \pm 0.00	24.52 \pm 0.92 ^b

¹ Protein contents calculated using N factors values determined for each sample in the present work: 5.88, 5.88, 5.92, and 5.87 for BR501(T-), BRS310(T-), BRS305(T+) and SC782(T+), respectively.

² Calculated by difference.

³ Values not detected with the given analysis conditions.

* Values in the same column with different letters are significantly different (ANOVA with Tukey test: $p < 0.05$). All experiments were carried out in triplicate.

Espinosa-Ramírez and Serna-Saldívar (2016), who also used the same extraction methodology, reported protein contents ranging from 72.7 to 88.6 g/100 g (d.b.) for extracts from whole sorghum grains. These results align with the findings of the present study, although the protein contents are lower than previously reported, especially for tannin-rich cultivars. It should be pointed out that the protein content in the extract depends not only on the cultivar and the extraction methodology, but also on the used N conversion factor. A generalized value of 6.25 is commonly used by manufacturers/suppliers of plant protein ingredients, including the works cited above, but this value is usually higher than the real values for plant proteins, leading to an overestimation of the protein content in such ingredients. For instance, Sosulski and Imafidon (1990) studied the amino acid composition of sorghum grain proteins and determined a N conversion factor of 5.93. In the present work, after analyzing the amino acid composition (Supplementary Table 1) and correlating the results with the protein sequences, specific N conversion factors were determined for each sample: 5.88 for BR501(T-) and BRS310(T-), 5.92 for BRS305(T+), and 5.87 for SC782(T+).

The lipid content in the flours ranged from 2.5 to 3.5 g/100 g (w.b.) (Table 1), with no significant differences between tannin-free and tannin-rich samples. Martino et al. (2012) reported lipid contents varying from 2.6 to 3.1 g/100 g (t.b.) for BR501(T-), BRS310(T-), and BRS305(T+) genotypes, aligning closely with the results in the present study. In the protein extracts, lipid content was noticeably high (18.7–26 g/100 g, t.b.), with the tannin-rich samples BRS305(T+) and SC782(T+) exhibiting the highest and lowest values, respectively. Espinosa-Ramírez and Serna-Saldívar (2016) obtained lipid contents ranging between 17.9 and 20.3 g/100 g (d.b.), whereas Da Silva and Taylor (2004) reported values of 12.8–16.7 g/100 g (d.b.) for protein-rich fractions. It is worth noting that the latter authors employed a Soxhlet extraction method, known as a lengthy process and involving hot solvent, which may have degraded some lipid components. Additionally, the Soxhlet method does not allow for a quantitative extraction of polar lipids, as discussed by Li et al. (2014). The high lipid contents observed in the protein extracts highlight that the applied extraction process not only concentrates proteins, but also results in a marked accumulation of the lipids initially present in the flours, which can be related to the fact that the flours were not defatted.

The water content was comparatively higher in the flours than in the respective protein extracts, whereas the ash content showed no significant difference between the two (Table 1). This observation can probably be attributed to the freeze-drying process used to prepare the

protein extracts. Regarding the carbohydrate content (i.e., the sum of the starch and fiber contents), higher values were obtained in the present study compared to the previous work of Martino et al. (2012), especially for tannin-rich cultivars. These disparities in proximate composition, even for the same cultivars, can be explained by variations in the cultivation and storage conditions of the grains.

In terms of total flavanols, encompassing flavanol monomers (catechin and epicatechin) and procyanidin oligomers and polymers (i.e., condensed tannins), the tannin-free samples BR501(T-) and BRS310(T-) did not contain any flavanols. In contrast, flavanols were present in the tannin-rich samples BRS305(T+) and SC782(T+). Interestingly, the proportions of flavanols in both the flours and protein extracts were of the same order of magnitude, ranging from 1.3 to 2.1 %. Consequently, no substantial enrichment of tannins in the extracts was observed compared to the initial flours. These findings may hold important implications for the functionality of the ingredients, as flavonoids such as flavanols have been recognized for their health benefits and antioxidant properties (Hackman et al., 2008).

3.1.2. Lipid composition of the powder samples

The accumulation of lipids in the sorghum protein extracts can be of high importance for the properties and functionality of these ingredients, and therefore a detailed analysis of the composition of the lipid extracts was conducted. Examples of the chromatograms obtained for the analysis of lipid classes, fatty acid composition and tocopherol content are shown in Supplementary Fig. 2.

No phospholipids (i.e., polar lipids) were detected in any sample. The predominant lipid class identified was free fatty acids (FFAs) (Fig. 1A), ranging from 51.3 to 75.8 % of the total lipids, followed by triacylglycerols (TAGs), ranging from 21.4 to 44.6 %. An exception was observed in the BR501(T-) flour, where TAGs (54.1 %) surpassed FFAs (41.5 %). All the flour samples were significantly different from each other regarding FFA and TAG contents, without a clear effect of the tannins. In the protein extracts, where the lipid fraction is substantial (as discussed in Section 3.1.2.), (T-) samples significantly differed from the (T+) ones in FFA content, with the former exhibiting the highest values. This suggests that the presence of tannins could be linked to a lower FFA content. Osagie (1987) and Price and Parsons (1975) reported that sorghum grains contained a small quantity of phospholipids and glycolipids, with neutral lipids being the predominant class (> 85 %), consistent with our study's findings. However, Osagie (1987) observed a large contribution of TAGs within neutral lipids (85 %), which was not found in the present study. The high FFA proportions present in our

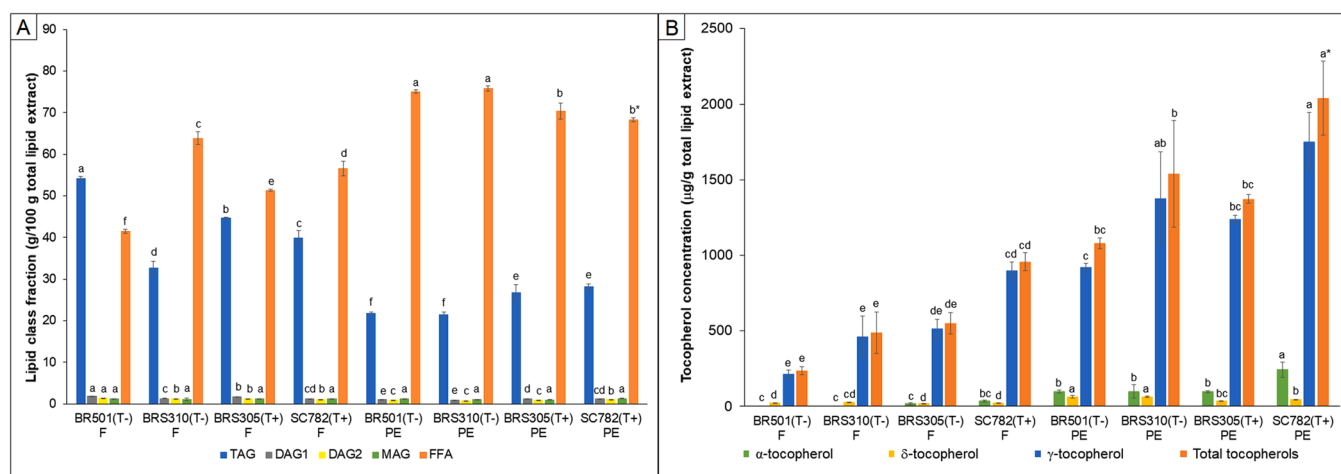


Fig. 1. Content in the different lipid classes (A) and tocopherol concentration (B) found in sorghum flours (suffix F) and their protein extracts (suffix PE). BR501(T-) and BRS310(T-) are the cultivars without endogenous tannins, whereas BRS305(T+) and SC782(T+) are the cultivars with tannins. TAG: triacylglycerol; DAG1: diacylglycerol-1–3; DAG2: diacylglycerol-1–2; MAG: monoacylglycerol; FFA: free fatty acid. *Different letters for the same lipid class or the same tocopherol isomer indicate significant differences (ANOVA with Tukey test: $p < 0.05$).

samples most likely result from the hydrolysis of TAGs initially present in the seeds, which may happen because of endogenous lipases either *in planta* (i.e., during the maturation of the seeds on the sorghum plants) or after harvesting of the seeds, for instance upon production or storage of the flours.

The fatty acid composition (Supplementary Table 2) of the sorghum lipids revealed a high concentration of linoleic acid (45–51 % of all fatty acids for the protein extracts, and 43–48 % for the flours), oleic acid (27–34 % for the protein extracts, and 29–37 % for the flours), and palmitic acid (13–15 % for the protein extracts and flours). This indicates a high content in PUFAs (> 50 % of all fatty acids) and MUFAs (> 30 %), rather than saturated FAs. A slight increase of linoleic acid and a slight decrease in oleic and palmitic acids were noted when comparing the fatty acid composition of the protein extracts with that of the initial flours, suggesting a slight accumulation of PUFAs during the protein extraction process. Hassan et al. (2017), Osagie (1987) and Price and Parsons (1975) obtained similar results for sorghum grains, where linoleic (43–58 %), oleic (21–37 %), and palmitic (13–19 %) acids were the main components identified in the fatty acid profile.

The total tocopherol content in the lipid extracts obtained from sorghum flours and their respective protein extracts ranged from 1371 to 2039 $\mu\text{g/g}$ total lipids. Tocopherol analysis (Fig. 1.B) revealed that the predominant component is γ -tocopherol, representing 85 to 94 % of the total tocopherol content. A substantial accumulation of tocopherols in the lipid fraction of the protein extracts was observed compared to the corresponding starting flour; the tocopherol content in the extracted lipid phase was multiplied by a factor of 2.1 to 4.6, depending on the sample, suggesting that the extraction process did not lead to a substantial chemical damage of these phytochemicals. Instead, it dramatically concentrated them in the final sample. Additionally, a higher tocopherol content was observed in the sample with a higher tannin content (SC782), both for flour and protein extract. These findings for flour samples align with the studies made by Martino et al. (2012), who reported similar values for tocopherol content, and predominantly γ -tocopherol. Li et al. (2021) also summarized tocopherol contents found in sorghum grains and observed the same pattern, having the highest values for γ -tocopherol.

3.1.3. FTIR spectra of the samples

The overall chemical fingerprint of the powder samples (flours and extracts), protein suspensions and their respective supernatants after centrifugation was first assessed by determining their infrared spectra (Supplementary Fig. 3.A and C). The compositional complexity of these samples is reflected in the infrared spectra, revealing multiple peaks corresponding to chemical groups typical of lipids (characterized by peaks at 2925 and 2854 cm^{-1} (methylene (-CH₂) and methyl (-CH₃) groups, respectively)), proteins (with peaks at 1652 and 1538 cm^{-1} (amide I (C=O) and amide II (N-H, C-N) groups, respectively)), and carbohydrates (with peaks at 3316.5 and 1151 to 900 cm^{-1} (O-H, C-O, and C-C groups, respectively)) (Lin et al., 2021). All the identified peaks and functional groups for the flours and protein extracts in this study align with the results obtained by Lin et al. (2021) and Xiao et al. (2015), respectively.

The secondary structure of proteins, correlated with the shape of the amide I band (Xiao et al., 2015) (Supplementary Fig. 3.B and D), was evaluated by performing a multiple peak fitting using a Gaussian function. Peaks ranging from 1620 to 1635 cm^{-1} , 1638 to 1640 cm^{-1} , 1645 to 1660 cm^{-1} , and 1670 to 16,790 cm^{-1} represent β -sheets, random coils, α -helices and β -turns, respectively. In the flours, the secondary structure was predominantly composed of α -helices (30–37 %) and β -sheets (22–31 %), with a minor presence of β -turns (17–24 %) and random coils (10–16 %). Conversely, protein extracts exhibited a greater contribution of β -sheets (31–34 %) compared to α -helices (27–29 %), with minor occurrence of β -turns (19–24 %) and random coils (8–9 %). When comparing a given flour and its respective protein extract, the presence of α -helices decreased within a range of 8–25 %. This suggests

that, in general, the extraction process had a substantial effect on the secondary structure of the proteins, especially for the cultivars containing tannins (BRS305 and SC782). This phenomenon could be attributed to the elevated temperature and reducing conditions (sodium metabisulfite) applied during the extraction process (Wang et al., 2009). In the aqueous suspensions, the secondary structure predominantly consisted of β -sheets (31–42 %) and α -helices (27–40 %), with some β -turns (1–15 %) and random coils (10–20 %), except for the SC782(T+) sample, which exhibited more α -helices (35–37 %) than β -sheets (15–26 %). Comparing the results before and after HPH, a noticeable decrease in α -helices and β -sheets was observed following this treatment, indicating a modification in the secondary structure of the proteins. This alteration might be attributed to the temperature increase during HPH (typical temperatures at the beginning and end of HPH were 20 and 45 °C,

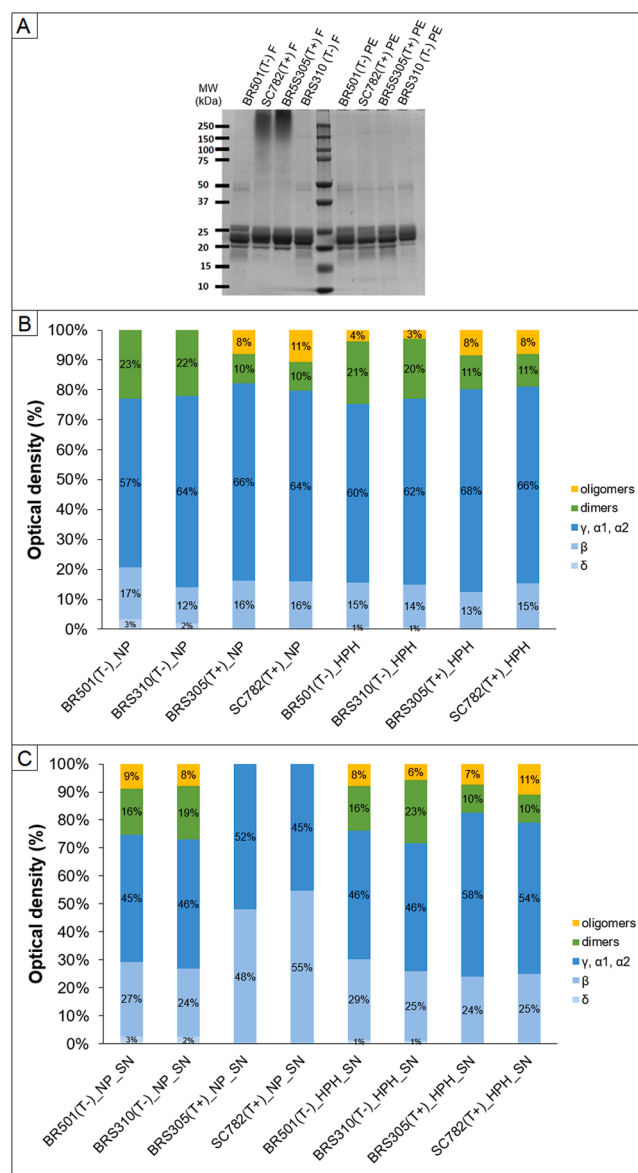


Fig. 2. Electrophoresis (SDS-PAGE) of sorghum proteins present in flours (suffix F) and their protein extracts (suffix PE) under reducing conditions (A), and protein composition determined by optical density analysis of SDS-PAGE gels of total suspensions (B), and their respective supernatants (C) (suffix SN), before (NP) and after homogenization (HPH). Samples were denatured and reduced by heating at 95 °C and using β -mercaptoethanol. BR501(T-) and BRS310(T-) are the cultivars without tannins, whereas BRS305(T+) and SC782(T+) are the cultivars with tannins.

respectively), coupled with enhanced hydration of the powder grains facilitated by intense shear and particle size reduction.

3.1.4. Protein composition of the samples

The protein types present in the sorghum flours from the different cultivars and in their respective protein extracts are illustrated in Fig. 2. A. Identifiable proteins include β -kafirin (20 kDa), α -kafirins (23 and 25 kDa) and γ -kafirin (28 kDa) across all samples. Dimers (approx. 50 kDa) were observed in the flours without tannins (BR501 and BRS310) and in all protein extracts, suggesting that the protein extraction process potentially induced protein aggregation in the samples with tannins (BRS305 and SC782). Notably, tannin-rich flours exhibited components with molecular weights exceeding 250 kDa, hinting at potential protein-tannin complexation. Large proteins with high molecular weight and flexible, open conformations have been shown to have affinity for tannins, a tendency that can be enhanced by protein denaturation (Butler, 1982; Rodrigues et al., 2009). The findings for the protein extracts align with studies performed by Belton et al. (2006), Espinosa-Ramírez and Serna-Saldívar (2016), Taylor et al. (2005), Wang et al. (2009) and Xiao et al. (2015), which demonstrated higher concentrations of α -kafirin compared to β - and γ -kafirins, dimers, trimers, and oligomers in various

sorghum protein extracts.

Upon analyzing the densitometry profiles of the SDS-PAGE gels for protein suspensions and their corresponding supernatants after centrifugation, the distribution of the different protein components was determined (Fig. 2.B and C). Minimal differences were observed between samples before and after HPH, with the protein composition mainly featuring β -kafirin (13–17 %), α - and γ -kafirins (57–68 %), dimers (10–23 %) and oligomers (3–11 %). In the samples without tannins (BR501(T-) and BRS310(T-)), some oligomers appeared after HPH, suggesting that the treatment may induce covalent aggregation. Notably, a distinction in protein components was observed between the total suspension (Fig. 2.B) and their respective supernatants (Fig. 2.C) for non-homogenized suspensions of tannin-rich samples. The former exhibited the presence of β -, α - and γ -kafirins, as well as dimers and oligomers, whereas only β -, α - and γ -kafirins were seen in the latter.

3.1.5. Size distribution of the protein components in the different flours and extracts

SE-HPLC was employed to analyze the molecular weight distribution and solubility of proteins (Fig. 3). For the flours, the SE-HPLC protein profiles of SDS-soluble proteins (SSP) revealed that the different

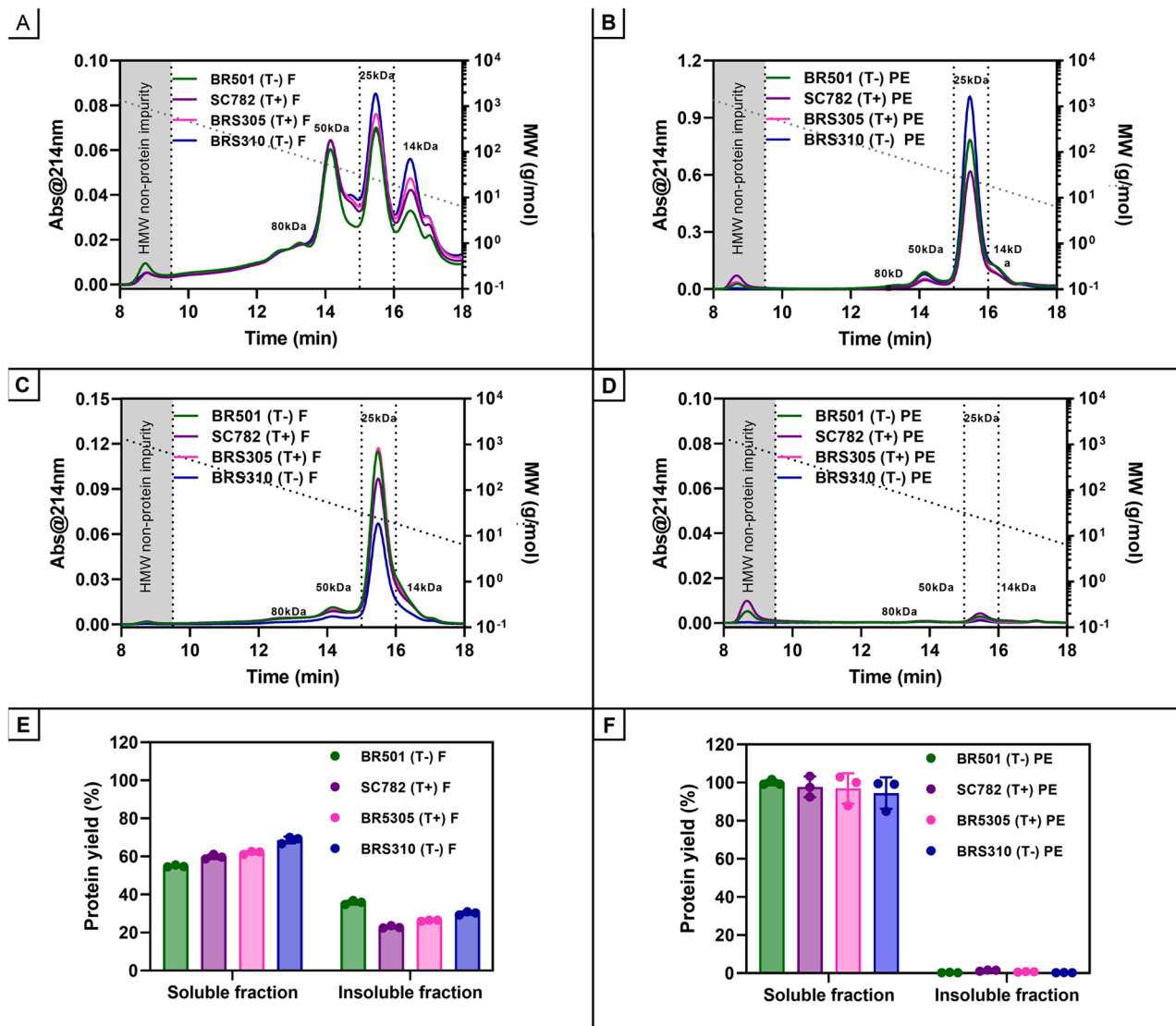


Fig. 3. Protein size distribution in sorghum flours and in their respective protein extracts for SDS-soluble (SSP) (A and B) and SDS-insoluble (SIP) (C and D) fractions; and protein quantification in the flours (E) and protein extracts (F) by size exclusion – high performance liquid chromatography (SE-HPLC). BR501(T-) and BRS310 (T-) are the cultivars without tannins, whereas BRS305(T+) and SC782(T+) are the cultivars with tannins.

genotypes, regardless of the presence or absence of tannins, exhibited a similar protein composition and size distribution. Kafirins monomers (all subclasses) were identified by the peak eluting around 25 kDa. However, the tannin-free genotype BR501(T⁻) displayed some differences, particularly in the peak around 14 kDa, where the intensity was lower (Fig. 3.A). The SE-HPLC profiles of SDS-insoluble proteins (SIP) (Fig. 3.C), primarily representing the cross-linked kafirins and glutelin fractions, looked fairly similar across different genotypes, although the kafirin peak (~25 kDa) differed in intensity. Within this fraction, an important reduction of polymeric proteins (peaks eluted around 50 and 80 kDa and beyond) occurred due to the reduction of disulfide bonds. The absence of the peak eluting around 14 kDa in the SIP for the extracts suggests that they correspond to non-kafirin proteins, probably small albumins and globulins.

In the protein extracts, all proteins were recovered in the SSP fraction (Fig. 3.B), predominantly as monomers eluting around 25 kDa. However, the presence of some polymeric kafirin forms (peaks at 50 and 80 kDa) was also observed. Only negligible amounts were recovered in the

SIP fraction (Fig. 3.D). When comparing the flours with their respective protein extracts, the SE-HPLC profile of the SIP from the flours mirrored that of the SSP from the protein extracts. This alignment is logical, considering the use of sodium metabisulfite (a reducing agent) during the protein extraction process.

Regarding quantitative aspects (Fig. 3.E and F), the amounts of SSP were higher than the SIP ones. The results unveil the presence of non-extractable protein fractions in the flour samples, in varying proportions (9 to 17 % of the total proteins) depending on the genotype. Similar results were reported by D'Almeida et al. (2021), who studied two Brazilian sorghum genotypes, one rich in tannins and the other tannin-free. Additionally, solubility differences were observed between (T⁺) and (T⁻) genotypes.

In conclusion, a robust agreement and complementarity were identified between the SE-HPLC and SDS-PAGE analyses (Section 3.1.4) concerning protein size distribution and identification.

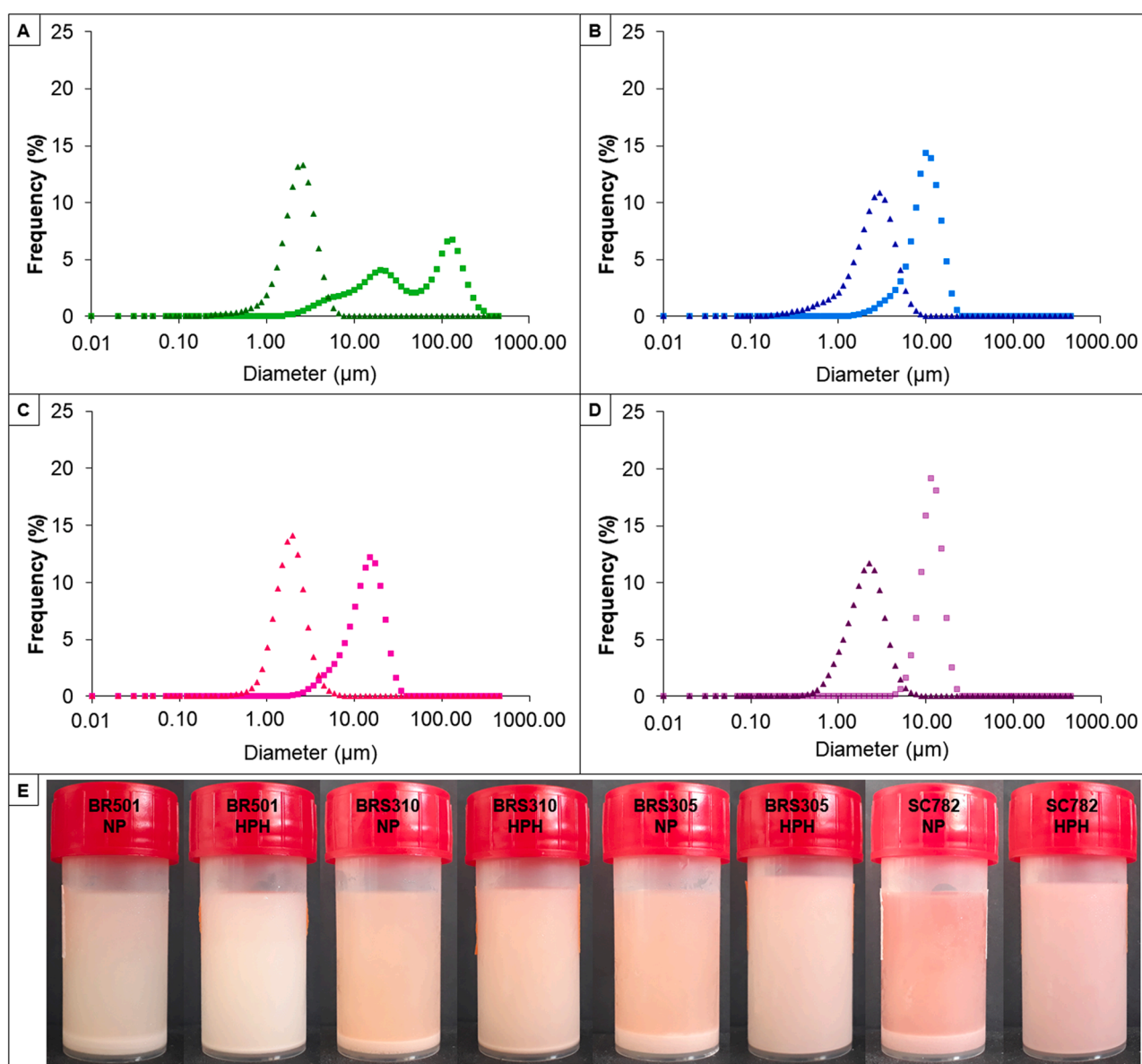


Fig. 4. Particle size distribution (distribution in volume frequency determined by static light scattering) in the suspensions before (square) and after (triangles) high pressure homogenization (HPH): (A) BR501(T⁻), (B) BRS310(T⁻), (C) BRS305(T⁺), (D) SC782(T⁺); and (E) macroscopic images of the suspensions before (NP) and after (HPH) homogenization. Pictures were taken 24 h after suspension preparation (and homogenization, when applicable).

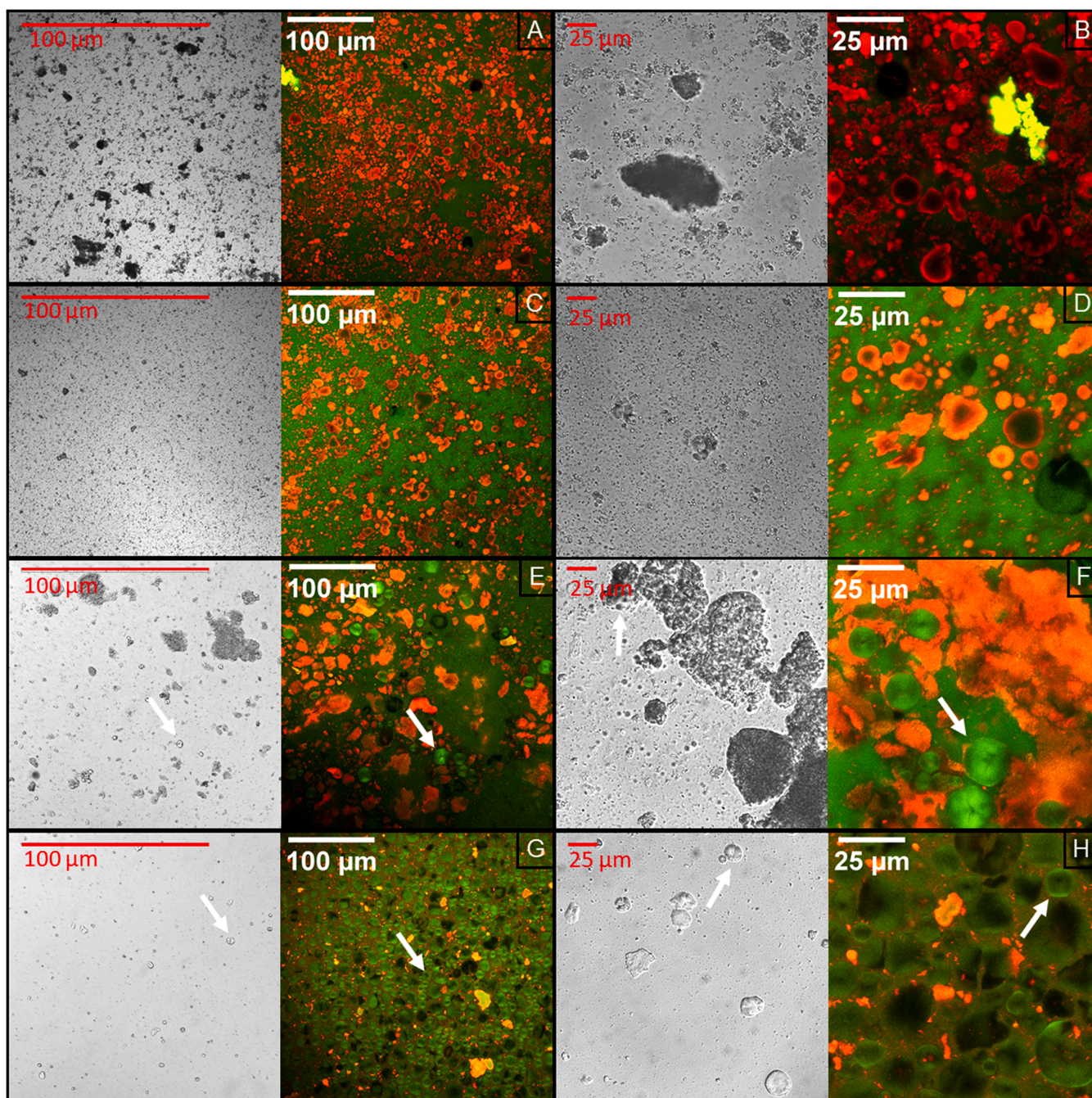


Fig. 5. Optical microscopy and CLSM images of two sorghum protein suspensions: (A, B) BR501(T-) before HPH, (C, D) BR501(T-) after HPH, (E, F) SC782(T+) before HPH, and (G, H) SC782(T+) after HPH. Proteins were stained with fast green and are visualized in green, whereas lipids were stained with Nile red and are visualized in red. White arrows indicate starch granules.

2.3. Behavior of the protein extracts in aqueous suspensions

3.2.1. Colloidal morphology of the protein suspensions

The particle size distributions of all aqueous suspensions of protein extracts, both before and after HPH (Fig. 4), demonstrated that HPH decreased the average particle size for all samples (initially ranging from 9 to 66 μm , and decreasing to 1.8 to 2.5 μm after the treatment). This effect was particularly pronounced in the suspension of the BR501(T-) protein extract (Fig. 4.A). The shear-based treatment also resulted in unimodal particle size distributions, instead of bimodal ones in the non-homogenized suspensions. Macroscopic images of the suspensions (Fig. 4.E) illustrated that HPH mitigated particle sedimentation at the

bottom of the tubes, especially in the samples with tannins (BRS305 and SC782). This is directly linked to the decrease in particle size, as per the Stokes law, where the rate of gravitational separation scales with the square of the particle size in dilute media (McClements, 2015).

The microstructure of the suspensions was evaluated by optical microscopy and CLSM images (Fig. 5), revealing the persistence of some large structures/aggregates (up to tens of μm) with irregular shapes even after HPH treatment. In the CLSM images, the presence of lipids (stained in red) was observed, which was expected as the lipid content in the protein extracts ranged between 18 and 26 % (g/100 g, t.b.). For the tannin-rich samples BRS305(T+) and SC782(T+) (Figs. 5.E-H), spherical, smooth structures were observed (white arrows), especially after

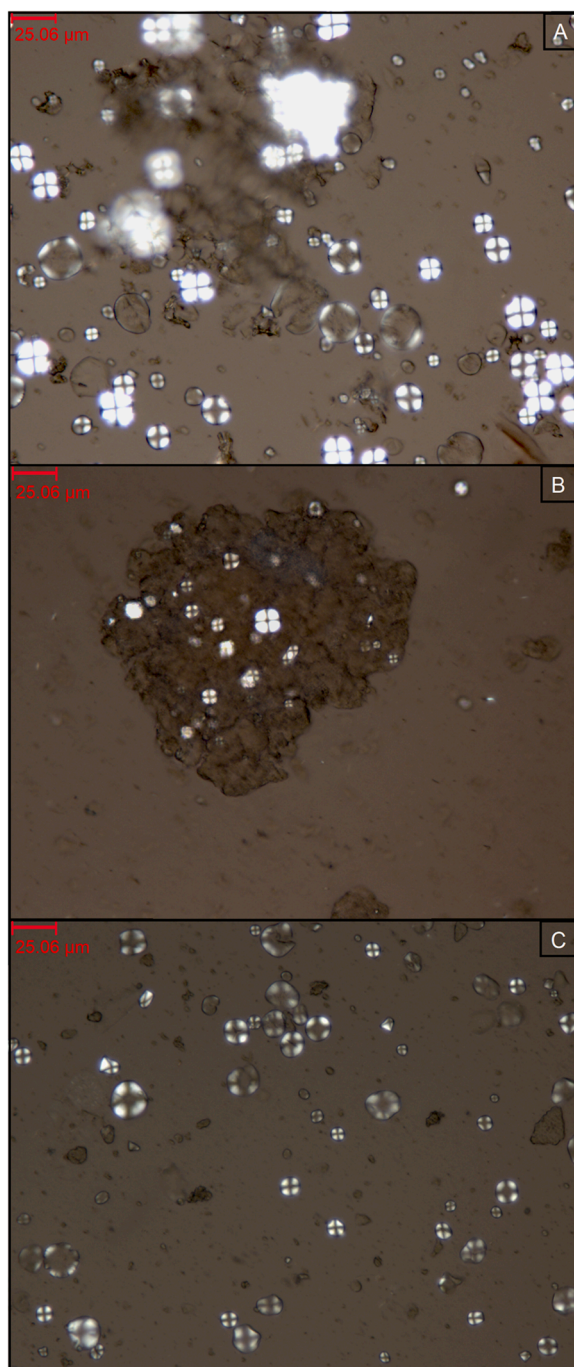


Fig. 6. Polarized light microscopy images for the SC782(T+)-based suspensions: (A) flour, (B) protein suspension before HPH, and (C) protein suspension after HPH.

HPH treatment, identified as starch granules through polarized light microscopy (Fig. 6). The SC782(T+) sample exhibited a substantial amount of starch granules in the flour (Fig. 6.A), which markedly decreased in the protein extract suspension (Fig. 6.B and C). This confirms that the extraction process effectively removed most of the starch. Additionally, the remaining starch granules were predominantly trapped within large composite structures following the extraction process (Fig. 6.B), probably protein- and/or fiber-based aggregates. The HPH treatment facilitated the release of these starch granules into the aqueous medium (Fig. 6.C).

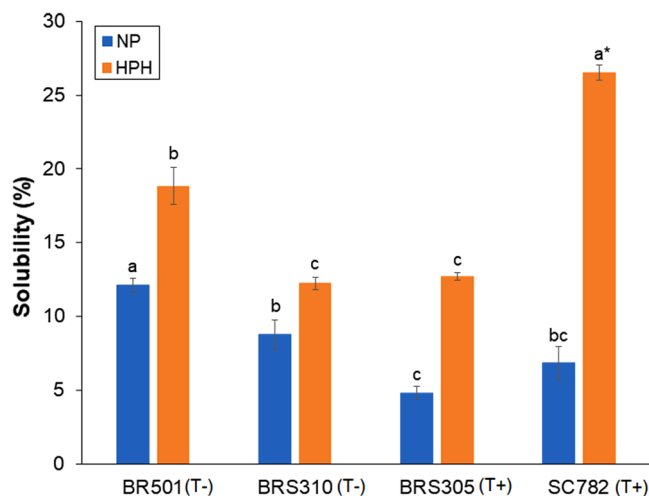


Fig. 7. Solubility (defined as the fraction of proteins that remained in the supernatant after centrifugation, compared to the total protein content in the suspension) of sorghum proteins in aqueous suspensions (ultrapure water) before and after treatment by HPH (suspensions were homogenized for 6 min at 400 bars). NP: non-treated by high pressure homogenization; HPH: treated by high pressure homogenization.

*Different letters for the same treatment indicate significant differences (ANOVA with Tukey test; $p < 0.05$).

3.2.2. Protein solubility in the aqueous suspensions

Conventionally, ‘solubility’ is defined as the fraction of proteins remaining in the supernatant of a given sample after centrifugation under specified conditions. However, these supernatants may contain small particles that do not sediment under these conditions, and that are often referred to as ‘soluble aggregates’. For simplicity, the terms ‘solubility’ and ‘soluble’ will be used in the following discussion, but one should bear in mind that this terminology may deviate slightly from the strict definition of solubility.

Protein solubility was assessed both before and after HPH treatment (Fig. 7). Homogenization substantially increased nitrogen concentration, and thus protein content in the supernatant, resulting in solubilities rising from 4.8–12.1 % to 12.2–26.5 % (a 1.4- to 3.9-fold increase, depending on the sample), especially in samples rich in tannins. This underscores the effectiveness of HPH for this purpose, suggesting promising technological improvements for handling such suspensions and unleashing their use for various applications. This observation aligns with the visual inspection of the samples before and after HPH (Fig. 4.E), where sedimentation was largely prevented by HPH treatment. Moll et al. (2021) applied Microfluidizer-based HPH on pea protein suspensions and observed a decrease in particle size and an increase in solubility (also defined as the ratio between nitrogen concentration in the supernatant and in the aqueous protein suspension). This confirms the potential of HPH to improve the processability of poorly soluble protein ingredients. A similar conclusion was recently drawn by Keuleyan et al. (2023), who found that HPH was helpful to improve solubility of various plant protein ingredients from pea and lupin. This was especially marked for highly processed ingredients, such as a pea protein isolate, characterized by a high endogenous lipid accumulation and poor initial solubility.

4. Conclusions

In this study, a detailed analysis of the composition of sorghum flours from tannin-poor or tannin-rich varieties, along with their derived protein extracts, was carried out. The protein extraction procedure proved efficient to yield high protein contents, especially for sorghum varieties without tannins. Besides achieving its primary objective of producing protein-rich ingredients, the extraction process also resulted

in a large accumulation of lipids in the extracts. The majority of these lipids were identified as free fatty acids (FFAs), mainly composed of linoleic, oleic, and palmitic acids. Additionally, the lipid fraction exhibited a remarkably high content in tocopherols. The substantial presence of lipids, especially FFAs, in sorghum protein ingredients may be of importance, particularly for their interfacial properties, which could influence the stability of emulsions produced with such ingredients.

As plant proteins are not well-soluble in aqueous media, which is a major limitation to their widespread use in food applications, high pressure homogenization (HPH) of the aqueous suspensions prepared with the protein extracts emerged as a potential means to enhance their dispersibility and protein solubility, especially for sorghum varieties with tannins. Consequently, HPH proves to be a valuable strategy to improve the processability of poorly soluble protein ingredients such as prolamin-rich fractions, and more broadly, various plant protein ingredients.

Ethical statement

This research did not involve human subjects nor animal experiments.

CRediT authorship contribution statement

Thais Cristina Benatti Gallo: Writing – original draft, Visualization, Investigation, Formal analysis. **Valérie Beaumal:** Writing – review & editing, Resources, Data curation. **Bérénice Houinsou-Houssou:** Writing – review & editing, Resources, Data curation. **Michèle Viau:** Writing – review & editing, Investigation. **Lucie Ribourg-Birault:** Writing – review & editing, Investigation. **Hélène Sotin:** Writing – review & editing, Investigation. **Joëlle Bonicel:** Investigation. **Adeline Boire:** Writing – review & editing, Resources. **Valéria Aparecida Vieira Queiroz:** Writing – review & editing, Resources. **Hamza Mameri:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Sylvain Guyot:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Alain Riaublanc:** Writing – review & editing, Formal analysis. **Vânia Regina Nicoletti:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Claire Berton-Carabin:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2024.100323.

References

- Ackman, R.G., 2007. Application of gas-liquid chromatography to lipid separation and analysis: qualitative and quantitative analysis. In: Chow, C.K. (Ed.), *Fatty Acids in Foods and Their Health Implications*. CRC Press, pp. 47–65.
- Antunes, R.C., Rodriguez, N.M., Gonçalves, L.C., Rodrigues, J.A.S., Borges, I., Borges, A. L.C.C., Saliba, E.O.S., 2007. Composição bromatológica e parâmetros físicos de grãos de sorgo com diferentes texturas do endosperma. *Arq. Bras. Med. Veterinária e Zootec.* 59, 1351–1354. <https://doi.org/10.1590/s0102-09352007000500042>.
- AOAC, 2006. *Official Methods of Analysis*, 18th ed. Association of Official Analytical Chemists, Gaithersburgs, MD.
- Bader, S., Bez, J., Eisner, P., 2011. Can protein functionalities be enhanced by high-pressure homogenization? – A study on functional properties of lupin proteins. *Procedia Food Sci* 1, 1359–1366. <https://doi.org/10.1016/j.profoo.2011.09.201>.
- Balny, C., Masson, P., 1993. Effects of high pressure on proteins. *Food Rev. Int.* 9, 611–628. <https://doi.org/10.1080/87559129309540980>.
- Barros, F., Awika, J., Rooney, L.W., 2014. Effect of molecular weight profile of sorghum proanthocyanidins on resistant starch formation. *J. Sci. Food Agric.* 94, 1212–1217. <https://doi.org/10.1002/jsfa.6400>.
- Belton, P.S., Delgadillo, I., Halford, N.G., Shewry, P.R., 2006. Kafirin structure and functionality. *J. Cereal Sci.* 44, 272–286. <https://doi.org/10.1016/j.jcs.2006.05.004>.
- Burger, T.G., Zhang, Y., 2019. Recent progress in the utilization of pea protein as an emulsifier for food applications. *Trend. Food Sci. Technol.* 86, 25–33. <https://doi.org/10.1016/j.tifs.2019.02.007>.
- Butler, L.G., 1982. Polyphenols and their effects on sorghum quality. In: *International Symposium on Sorghum Grain Quality*. Patancheru, India, pp. 291–311.
- D'Almeida, C.T.dos S., Mameri, H., Menezes, N.dos S., de Carvalho, C.W.P., Queiroz, V.A.V., Cameron, L.C., Morel, M.H., Takeiti, C.Y., Ferreira, M.S.L., 2021. Effect of extrusion and turmeric addition on phenolic compounds and kafirin properties in tannin and tannin-free sorghum. *Food Res. Int.* 149 <https://doi.org/10.1016/j.foodres.2021.110663>.
- Da Silva, L.S., Taylor, J.R.N., 2004. Sorghum bran as a potential source of kafirin. *Cereal Chem* 81, 322–327. <https://doi.org/10.1094/CCHEM.2004.81.3.322>.
- de Mesa-Stonestreet, N.J., Alavi, S., Bean, S.R., 2010. Sorghum proteins: the concentration, isolation, modification, and food applications of kafirins. *J. Food Sci.* 75 <https://doi.org/10.1111/j.1750-3841.2010.01623.x>.
- Duodu, K.G., Taylor, J.R.N., Belton, P.S., Hamaker, B.R., 2003. Factors affecting sorghum protein digestibility. *J. Cereal Sci.* 38, 117–131. [https://doi.org/10.1016/S0733-5210\(03\)00016-X](https://doi.org/10.1016/S0733-5210(03)00016-X).
- Espinosa-Ramírez, J., Serna-Saldívar, S.O., 2016. Functionality and characterization of kafirin-rich protein extracts from different whole and decorticated sorghum genotypes. *J. Cereal Sci.* 70, 57–65. <https://doi.org/10.1016/j.jcs.2016.05.023>.
- FAO, 2003. *Food Energy – Methods of Analysis and Conversion Factors*. FAO Food and Nutrition Paper 77 [WWW Document]. URL <https://www.fao.org/3/Y5022E/Y5022E00.htm> (accessed 12.20.23).
- FAOSTAT, 2022. *Food and Agriculture Organization of the United Nations*. FAO. <https://www.fao.org/faostat/en/?#data/QCL> (accessed 12.13.22).
- Fogang Mba, A.R., Kansci, G., Viau, M., Hafnaoui, N., Meynier, A., Demmano, G., Genot, C., 2017. Lipid and amino acid profiles support the potential of *Rhynchophorus phoenicis* larvae for human nutrition. *J. Food Compos. Anal.* 60, 64–73. <https://doi.org/10.1016/J.JFCA.2017.03.016>.
- Fogang Mba, A.R., Kansci, G., Viau, M., Ribourg, L., Muafor, J.F., Hafnaoui, N., Gall, P.L., Genot, C., 2018. Growing conditions and morphotypes of African palm weevil (*Rhynchophorus phoenicis*) larvae influence their lipophilic nutrient but not their amino acid compositions. *J. Food Compos. Anal.* 69, 87–97. <https://doi.org/10.1016/j.jfca.2018.02.012>.
- Girard, A.L., Awika, J.M., 2018. Sorghum polyphenols and other bioactive components as functional and health promoting food ingredients. *J. Cereal Sci.* 84, 112–124. <https://doi.org/10.1016/j.jcs.2018.10.009>.
- Hackman, R.M., Polagruto, J.A., Zhu, Q.Y., Sun, B., Fujii, H., Keen, C.L., 2008. Flavanols: digestion, absorption and bioactivity. *Phytochem. Rev.* 7, 195–208. <https://doi.org/10.1007/s11101-007-9070-4>.
- Hassan, S., Imran, M., Ahmad, N., Khan, M.K., 2017. Lipids characterization of ultrasound and microwave processed germinated sorghum. *Lipid. Health Dis.* 16, 1–11. <https://doi.org/10.1186/s12944-017-0516-4>.

- Keuleyan, E., Gélébart, P., Beaumal, V., Kermaerrec, A., Ribourg-Birault, L., Le Gall, S., Meynier, A., Riaublanc, A., Berton-Carabin, C., 2023. Pea and lupin protein ingredients: new insights into endogenous lipids and the key effect of high-pressure homogenization on their aqueous suspensions. *Food Hydrocoll.* 141 <https://doi.org/10.1016/j.foodhyd.2023.108671>.
- Levy, R., Okun, Z., Shpigelman, A., 2022. Utilizing high-pressure homogenization for the production of fermented plant-protein yogurt alternatives with low and high oil content using potato protein isolate as a model. *Innov. Food Sci. Emerg. Technol.* 75, 102909 <https://doi.org/10.1016/j.ifset.2021.102909>.
- Li, Y., Ghasemi Naghdi, F., Garg, S., Adarme-Vega, T.C., Thurecht, K.J., Ghafor, W.A., Tannock, S., Schenk, P.M., 2014. A comparative study: the impact of different lipid extraction methods on current microalgal lipid research. *Microb. Cell Fact.* 13, 1–9. <https://doi.org/10.1186/1475-2859-13-14>.
- Li, Z., Zhao, X., Zhang, X., Liu, H., 2021. Bioactive compounds and biological activities of sorghum grains. *Foods* 10, 1–20. <https://doi.org/10.3390/foods10112868>.
- Lin, H., Bean, S.R., Tilley, M., Peiris, K.H.S., Brabec, D., 2021. Qualitative and quantitative analysis of sorghum grain composition including protein and tannins using ATR-FTIR spectroscopy. *Food Anal. Method.* 14, 268–279. <https://doi.org/10.1007/s12161-020-01874-5>.
- Liu, L., Chen, L., Abbasi, A.M., Wang, Z., Li, D., Shen, Y., 2018. Optimization of extraction of polyphenols from Sorghum Moench using response surface methodology, and determination of their antioxidant activities. *Trop. J. Pharm. Res.* 17, 619–626. <https://doi.org/10.4314/tjpr.v17i4.8>.
- Malec, M., Le Quééré, J.M., Sotin, H., Kolodziejczyk, K., Bauduin, R., Guyot, S., 2014. Polyphenol profiling of a red-fleshed apple cultivar and evaluation of the color extractability and stability in the juice. *J. Agric. Food Chem.* 62, 6944–6954. <https://doi.org/10.1021/jf500336v>.
- Martino, H.S.D., Tomaz, P.A., Moraes, É.A., Conceição, L.L.da, Oliveira, D.da S., Queiroz, V.A.V., Rodrigues, J.A.S., Pirozi, M.R., Pinheiro-Sant'Ana, H.M., Ribeiro, S.M.R., 2012. Chemical characterization and size distribution of sorghum genotypes for human consumption. *Rev. Inst. Adolfo Lutz* 71, 337–344.
- McClements, D.J., 2015. *Food Emulsions: Principles, Practices, and Techniques*, 3rd ed. CRC Press. <https://doi.org/10.1201/b18868>.
- Mehmood, S., Orhan, I., Ahsan, Z., Aslan, S., Gulfranz, M., 2008. Fatty acid composition of seed oil of different Sorghum bicolor varieties. *Food Chem.* 109, 855–859. <https://doi.org/10.1016/j.foodchem.2008.01.014>.
- Melchior, S., Moretton, M., Calligaris, S., Manzocco, L., Nicoli, M.C., 2022. High pressure homogenization shapes the techno-functionalities and digestibility of pea proteins. *Food Bioprod. Process.* 131, 77–85. <https://doi.org/10.1016/j.fbp.2021.10.011>.
- Meynier, A., Leborgne, C., Viau, M., Schuck, P., Guichardant, M., Rannou, C., Anton, M., 2014. N-3 fatty acid enriched eggs and production of egg yolk powders: an increased risk of lipid oxidation? *Food Chem.* 153, 94–100. <https://doi.org/10.1016/j.foodchem.2013.12.028>.
- Moll, P., Salminen, H., Schmitt, C., Weiss, J., 2021. Impact of microfluidization on colloidal properties of insoluble pea protein fractions. *Eur. Food Res. Technol.* 247, 545–554. <https://doi.org/10.1007/s00217-020-03629-2>.
- Morel, M.H., Pincemaille, J., Lecacheux, L., Menut, P., Ramos, L., Banc, A., 2022. Thermodynamic insights on the liquid-liquid fractionation of gluten proteins in aqueous ethanol. *Food Hydrocoll.* 123 <https://doi.org/10.1016/j.foodhyd.2021.107142>.
- Morrison, W.R., Smith, L.M., 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* 5, 600–608. [https://doi.org/10.1016/S0022-2275\(20\)40190-7](https://doi.org/10.1016/S0022-2275(20)40190-7).
- Neuhoff, V., Arold, N., Taube, D., Ehrhardt, W., 1988. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis* 9, 255–262. <https://doi.org/10.1002/elps.1150090603>.
- Osagie, A.U., 1987. Total lipids of sorghum grain. *J. Agric. Food Chem.* 35, 601–604. <https://doi.org/10.1021/jf00076a038>.
- Price, P.B., Parsons, J.G., 1975. Lipids of seven cereal grains. *J. Am. Oil Chem. Soc.* 52, 490–493. <https://doi.org/10.1007/BF02640738>.
- Proietti, I., Frazzoli, C., Mantovani, A., 2015. Exploiting nutritional value of staple foods in the world's semi-arid areas: risks, benefits, challenges and opportunities of sorghum. *Healthcare* 3, 172–193. <https://doi.org/10.3390/healthcare3020172>.
- Queiroz, V.A.V., Schaffert, R.E., Moreira, A.V., Machado, S., Ribeiro, R., Duarte, S., 2011. O sorgo. *Rev. Bras. Milho e Sorgo* 10, 180–195.
- Rodrigues, W.A., Magalhães, P.C., Paiva, E., dos Santos, F.G., 2009. Padrões eletroforéticos das proteínas de reserva em grãos de sorgo com presença e ausência de tanino. *Cienc. e Agrotecnologia* 33, 1972–1977. <https://doi.org/10.1590/s1413-70542009000700047>.
- Sosulski, F.W., Imafidon, G.I., 1990. Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. *J. Agric. Food Chem.* 38, 1351–1356. <https://doi.org/10.1021/jf00096a011>.
- Taylor, J., Taylor, J.R.N., Dutton, M.F., De Kock, S., 2005. Glacial acetic acid - A novel food-compatible solvent for kafirin extraction. *Cereal Chem.* 82, 485–487. <https://doi.org/10.1094/CC-82-0485>.
- Vallons, K.J.R., Ryan, L.A.M., Koehler, P., Arendt, E.K., 2010. High pressure-treated sorghum flour as a functional ingredient in the production of sorghum bread. *Eur. Food Res. Technol.* 231, 711–717. <https://doi.org/10.1007/s00217-010-1316-5>.
- Wang, Y., Tilley, M., Bean, S., Susan Sun, X., Wang, D., 2009. Comparison of methods for extracting kafirin proteins from sorghum distillers dried grains with solubles. *J. Agric. Food Chem.* 57, 8366–8372. <https://doi.org/10.1021/jf901713w>.
- Xiao, J., Chen, Y., Huang, Q., 2017. Physicochemical properties of kafirin protein and its applications as buildings blocks of functional delivery systems. *Food Funct.* 8, 1402–1413. <https://doi.org/10.1039/C6FO01217E>.
- Xiao, J., Li, Y., Li, J., Gonzalez, A.P., Xia, Q., Huang, Q., 2015. Structure, morphology, and assembly behavior of kafirin. *J. Agric. Food Chem.* 63, 216–224. <https://doi.org/10.1021/jf504674z>.
- Zamora, A., Guamis, B., 2015. Opportunities for ultra-high-pressure homogenisation (UHPH) for the food industry. *Food Eng. Rev.* 7, 130–142. <https://doi.org/10.1007/s12393-014-9097-4>.